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Sensitivity of eucalyptus (*Eucalyptus urograndis*) plants to subdoses of the herbicide dicamba

Sensibilidade de plantas de eucalipto (Eucalyptus urograndis) à subdoses do herbicida dicamba

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ABSTRACT

In view of the widespread increase in herbicide-resistant weeds, biotechnology companies have developed dicamba-tolerant soybean and cotton cultivars. This technology can, however, increase the risk of the product drifting to adjacent areas. This study was developed with the objective of the to evaluate the phytotoxicity and biometric variables of young eucalyptus plants exposed to subdoses of the herbicide dicamba. The experiment was carried out under field conditions in Rio Verde, state of Goiás, Brazil. The treatments were represented by the application of 0 (control), 7.5, 15, 30, 60, 120 or 240 g ae ha^{-1} of dicamba 45 days after the seedlings were planted in the field. In terms of phytotoxicity, the dicamba doses of 120 and 240 g ae ha^{-1} caused greater damage to the eucalyptus plants in all periods of evaluation. The predominant symptoms were epinasty, increased number of shoots and necrosis and senescence of young branches and leaves. The herbicide doses of 120 and 240 g ae ha^{-1} significantly compromised plant height and diameter, number of branches and dry mass of leaves and roots, interfering with the growth and development of the eucalyptus crop. The results indicate that the effect of subdoses of the herbicide dicamba can interfere with the proper development of young eucalyptus plants, which may cause losses in the initial planting phase and future losses for producers.

KEYWORDS: Auxinic herbicide; phytotoxicity; reforestation; biomass production.

RESUMO

Em decorrência do aumento generalizado de plantas daninhas com resistência a herbicidas, empresas de biotecnologia desenvolveram cultivares de soja e algodão tolerantes ao herbicida dicamba. Essa tecnologia pode, no entanto, aumentar o risco do produto ser deslocado para áreas adjacentes às aplicadas. Neste trabalho objetivou-se avaliar a fitotoxicidade e variáveis biométricas de plantas jovens de eucalipto tratadas com subdoses do herbicida dicamba. O experimento foi realizado em condições de campo em Rio Verde, Goiás, Brasil. Os tratamentos foram representados pela aplicação de 0 (testemunha), 7,5, 15, 30, 60, 120 ou 240 g ea ha^{-1} de dicamba aos 45 dias após o plantio das mudas no campo. Em termos de fitotoxicidade, as doses de dicamba de 120 e 240 g ea ha^{-1} causaram maiores danos às plantas de eucalipto em todos os períodos de avaliação. Os sintomas predominantes foram epinastia, aumento do número de brotações e necrose e senescência de ramos e folhas jovens. As doses de herbicidas de 120 e 240 g ea ha^{-1} comprometeram significativamente a altura e diâmetro das plantas, número de ramos e massa seca de folhas, caules e raízes, interferindo no crescimento e desenvolvimento da cultura do eucalipto. Os resultados indicam que o efeito de subdoses do herbicida dicamba pode interferir no bom desenvolvimento de plantas jovens de eucalipto, podendo causar prejuízos na fase inicial de plantio e prejuízos futuros para os produtores.

PALAVRAS-CHAVE: Herbicida auxínico; fitotoxicidade; reflorestamento; produção de biomassa.

INTRODUCTION

The chemical control achieved with herbicide application constitutes an important measure of weed control in production systems (TIMOSSI & FREITAS 2011, SILVA et al. 2018). Glyphosate is the most prominent of all herbicides commercialized in Brazil, accounting for approximately 31.5% of the total (AGROFIT 2018). However, the repeated and improper use of this herbicide has resulted in the selection of resistant weeds, often making their control more difficult (OWEN 2016).

Therefore, research companies have sought new alternatives for weed control. One of such alternatives is the introduction of genetically modified crops such as soybean and cotton, which are tolerant to dicamba, an auxiliary herbicide used in post-emergence to control broadleaf weeds (KRUGER et al. 2010, MORTENSEN et al. 2012). In Brazil, the technology became commercially available in several soybean cultivars in the 2021/2022 harvest.

The herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) is a hormone growth regulator that belongs to the class of auxin-mimicking herbicides and the chemical group of benzoic acids. This is due to its functional and structural similarity to indoleacetic acid (IAA), the main natural hormone that regulates the activity of several genes involved in plant growth (ZHOU et al. 2016).

However, dicamba has been associated with volatility and displacement of product molecules to areas adjacent to those of application, which may cause damage to plants (EGAN et al. 2014, CUNDIFF et al. 2017, JOSEPH et al. 2018, GAZOLA et al. 2021, CANTU et al. 2021). With the introduction of this technology in the market, there is a greater chance of this herbicide drifting to unintended areas (VIEIRA et al. 2020).

MUELLER et al. (2015), reported that the number of articles in journals related to the “drift” and “herbicide” topics tripled between 1991 and 2012. This shows the importance and concern of researchers regarding the subject, since subdoses of the herbicide due to drift can result in significant environmental impacts and economic losses. For the eucalyptus crop, several studies have already shown the negative effects of the subdoses of different herbicides (TIBURCIO et al. 2012, SANTOS JÚNIOR et al. 2015, PEREIRA et al. 2015, SALGADO et al. 2017), but studies on dicamba are still rare.

Since the release of this technology in the USA, the agriculture departments of the States registered approximately 2,200 cases of suspected dicamba injuries in sensitive plants due to the drift process (BASF CORPORATION 2017, BRADLEY 2017). This is especially true for areas where integrated production systems are implemented, such as agrosilvopastoral system, where the eucalyptus crop has not been genetically modified to tolerate exposure to this herbicide and herbicide application is commonly adopted.

The use of eucalyptus for biomass production to meet the energy demand is consolidated in some regions of Brazil, especially the Center-West (SIMIONI et al. 2017). Within this region, the state of Goiás has stood out with significant growth in recent years in terms of areas of eucalyptus planted forests. In particular, the municipality of Rio Verde - GO is among the 20 largest producers of firewood in Brazil and energy utilization serves the industries in the region, mainly for the grain drying/processing and meat sectors (FORMOLO JUNIOR et al. 2019).

In this context, the present study was developed to evaluate and characterize the possible damage caused by the herbicide dicamba to young eucalyptus plants by analyzing phytotoxicity and biometric variables.

MATERIAL AND METHODS

The study was carried out under field conditions at Goiano Federal Institute, Rio Verde Campus - GO, Brazil (17°48'67" S, 50°54'18" W, 758 m above sea level). The soil in the experimental area is classified as dystrophic red Oxisol, and soil analysis performed at a depth of 0 to 20 cm revealed the following characteristics: pH 6.2 (SMP); 4.64 Ca, 2.50 Mg, 0.02 Al^{3+}, 3.04 H+Al, 12.1 CEC and 0.37 K ($cmol_c$ dm^{-3}); 10.28 mg dm^{-3} P (Melich); 3.62 dag kg^{-1} organic matter; and 62.8% base saturation. As for climatological data, the rainfall, relative humidity and average temperature recorded during the research period are presented in Figure 1.

Eucalyptus seedlings of approximately four months old, with an average height of 35 cm, were acquired from a registered nursery. The commercial clone used was I-144 (*Eucalyptus urograndis*), a hybrid resulting from the crossing of *E. urophylla* × *E. grandis*. On November 22, 2018, the seedlings were transplanted into open pits with dimensions of 20×20×20 cm. Time-of-planting fertilization consisted of the application of 100 g of NPK (4-30-16) per pit. Cultivation treatments such as the control of leaf-cutting ants were carried out in accordance with technical recommendations for the eucalyptus crop. Weeds were controlled manually, when necessary.

After the seedling-acclimatization period of 45 days, dicamba was applied using a backpack sprayer pressurized with CO_2 and regulated to obtain a constant pressure of 150 KPa, at a flow rate of 226 L ha^{-1}. The sprayed solution was applied directly to the eucalyptus leaves at 08h00. The spray nozzles used were the fan type, model JDF 02 Jacto. The meteorologic conditions at the time of spraying were: wind speed 1 m s^{-1}, temperature 25.5 °C and relative humidity 65%.

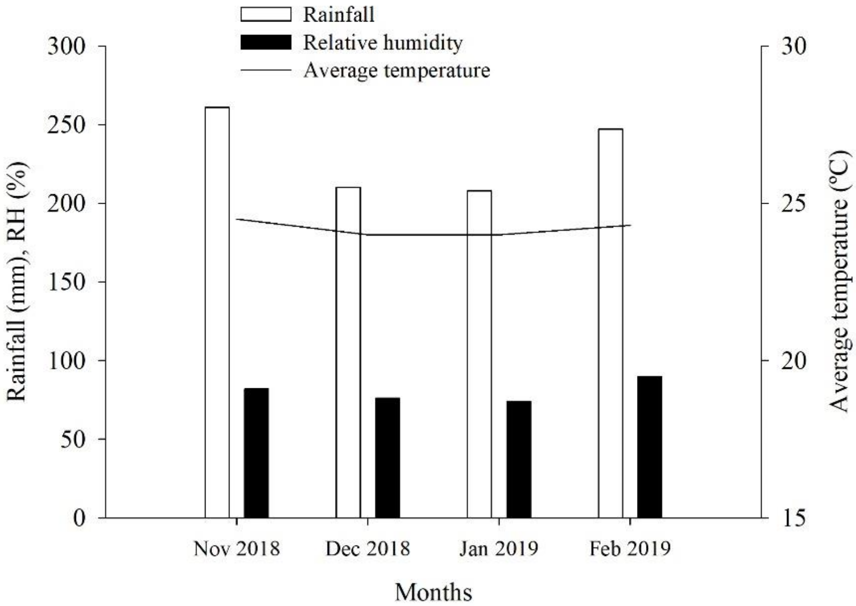

Figure 1. Rainfall, average temperature and relative humidity (RH) during the study period.

The experiment was laid out in a randomized-block design with five replicates and seven treatments, totaling 35 experimental plots. Each plot consisted of 12 eucalyptus plants. Plants were spaced 1 m apart, to maximize the experimental area. The treatments corresponded to different subdoses of dicamba, namely, 0 (check plots), 7.5, 15, 30, 60, 120 and 240 g ae ha^{-1}. Because a drift simulation was performed in this study, lower doses were chosen in relation to that recommended in the package insert (JOHNSON et al. 2012, SMITH et al. 2017).

Phytotoxicity symptoms were assessed at 7, 14, 21 and 28 days after application (DAA) based on visual observations and by assigning phytotoxicity grades of 0 to 100%, according to the EWRC scale, modified by FRANS (1972), as shown in Table 1. The grades were assigned by three evaluators, at all evaluation times, and the average percentage of each experimental plot was considered.

Table 1. Visual phytotoxicity scale used to evaluate the effect of subdoses of the dicamba herbicide on *Eucalyptus urograndis* plants.

Scale	Toxicity (%)	Toxicity characteristic
1	0	Null
2	1 – 3.5	Very mild
3	3.5 – 7.0	Mild
4	7.0 – 12.5	No impact on production
5	12.5 – 20.0	Medium
6	20.0 – 30.0	Almost strong
7	30.0 – 50.0	Strong
8	50.0 – 99.0	Very strong
9	100	Death of plants

Source: EWRC (European Weed Research Council), modified by Frans (1972).

In the biometric evaluation, height (cm) was measured using a millimeter ruler from the soil surface to the apex of the plant; and stem diameter (mm) was measured using a digital caliper approximately 2 cm from the soil surface. Four plants were evaluated per plot, selecting those located in the central row. These evaluations were performed prior to application, for comparison, and at 14 and 28 DAA.

At 90 DAA, the number of branches and the dry mass of branches and leaves were determined. Two plants per plot, located in the central row, were selected, segmented into the morphological parts and weighed. Aliquots of 500 g were placed in paper bags, which were then dried in a forced-air oven at a temperature of 65 ºC for three days, until reaching constant weight. The dry matter of certain parts was calculated based on the total weight of the plants as well as the fresh and dry matter weights of the aliquots.

Data were subjected to analysis of variance ($p \leq 0.05$) by the F test and to regression analysis thereafter. Statistical analyses were carried out using SISVAR® statistical software.

RESULTS AND DISCUSSION

In all evaluations, phytotoxicity symptoms in the eucalyptus plants increased linearly with the dicamba doses (Figure 2), but with a low angular coefficient, between 0.053 and 0.069. At the highest doses (120 and 240 g ae ha^{-1}), the symptoms did not exceed 20% phytotoxicity, which was thus considered medium. At lower doses (7.5, 15, 30 and 60 g ae ha^{-1}), the injury symptoms were considered null to mild, showing that, at these doses, there was no negative interference with the establishment of eucalyptus (Figure 2). Visual phytotoxicity is often related to the dose, where higher levels can intensify the symptoms.

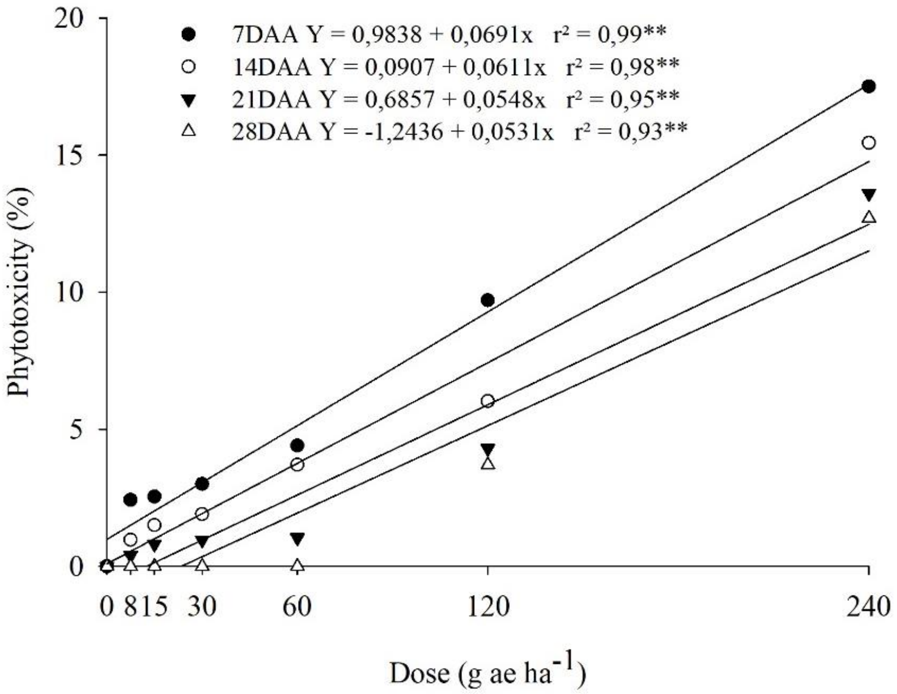

Figure 2. Phytotoxicity in young *Eucalyptus urograndis* plants after exposure to different subdoses of the herbicide dicamba, at 7, 14, 21 and 28 days after application (DAA).

At 7 DAA, the eucalyptus plants showed petiole elongation, epinasty of leaves, branches and stems and twisting of young tissues when the dose of 240 g ae ha^{-1} was applied (Figure 3). Similar results were obtained by TUFFI SANTOS et al. (2006), who evaluated different doses of triclopyr (14.4, 28.8 and 57.6 g ha^{-1}) in young eucalyptus plants. Similar symptoms were also observed in other crops subjected to dicamba subdoses, such as pea, common bean and potato (HATTERMAN-VALENTI et al. 2017), grapevine (MOHSENI-MOGHADAM et al. 2016) and soybean (SOLOMON & BRADLEY 2014).

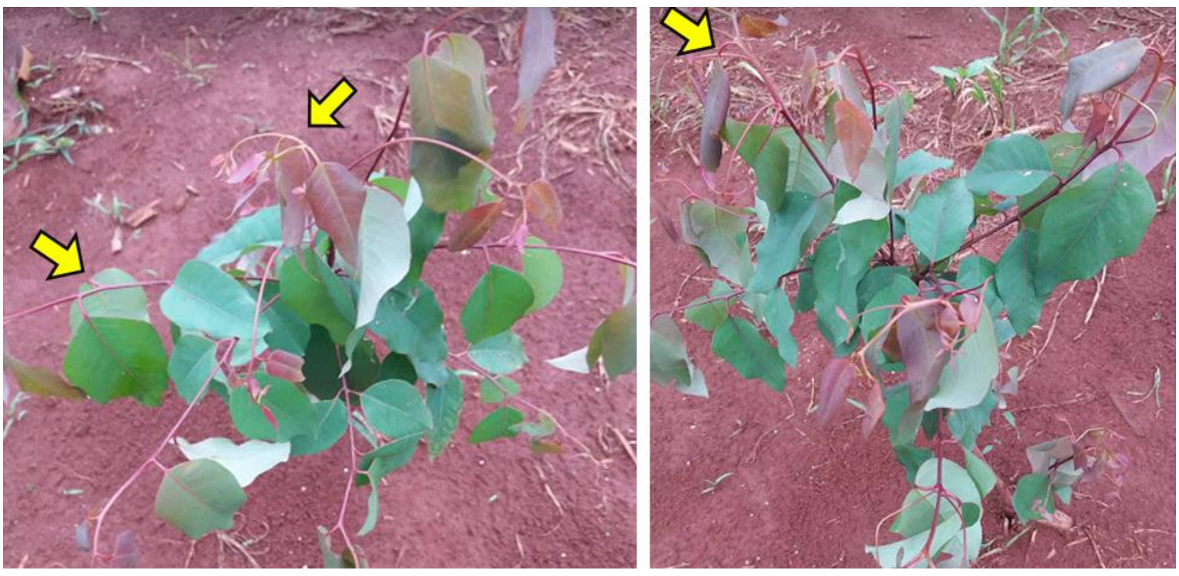

Figure 3. Leaf and branch epinasty symptoms after exposure to 240 g ae ha^{-1} of the herbicide dicamba in *Eucalyptus urograndis* seedlings at 7 DAA.

At 14 DAA, at the highest doses, the symptoms were the same as those observed on the previous evaluation date. In addition to these symptoms, the young branches and leaves showed signs of necrosis in the meristematic region (Figure 4). In a similar study, CARVALHO et al. (2014) reported similar symptoms after applying 25% of the recommended dose of the herbicide triclopyr and the formulated mixture of triclopyr + fluroxypyr in eucalyptus seedlings. According to SENSEMAN (2007), auxin-mimicking herbicides act to synthesize ethylene as cell division inhibitors, inducing symptoms of disorganized meristem growth, wilting, chlorosis, necrosis of leaves and branches, and, later, senescence.

Figure 4. Epinasty and necrosis symptoms after exposure to 240 g ae ha^{-1} of the herbicide dicamba in *Eucalyptus urograndis* seedlings at 14 DAA.

After 21 DAA, phytotoxicity ranged from 0 to 13.6% between the treatments. In the same period, shoots appeared in the stem region and branches and leaves showed necrosis (Figure 5). As stated by TUFFI SANTOS et al. (2005), shoot emergence is commonly seen in intoxicated plants in response to herbicide subdosage. Similar results were shown by CARVALHO et al. (2014), who described the appearance of interneval chlorosis and new shoots in the *E. urophylla* × *E. camaldulensis* hybrid after application of 50% of the recommended dose of fluroxypyr + triclopyr. In the same study, the authors observed the same symptoms when 25% of the recommended dose of triclopyr was applied to the hybrid *Eucalyptus urograndis*. According to TAIZ & ZEIGER (1998), synthetic auxins are regulators of apical dominance processes, and shoot emergence is related to cytokine production and release, implying axillary bud formation.

Figure 5. Symptoms of necrosis of young branches and leaves and shoot emergence after exposure to 240 g ae ha^{-1} of the herbicide dicamba in *Eucalyptus urograndis* seedlings at 21 DAA.

At 28 DAA, shoots appeared and there was a slight reduction in symptoms, showing that eucalyptus plants may have mechanisms that allow a gradual the recovery of injuries caused by exposure to the herbicide (Figure 6). Similar results were observed by CARVALHO et al. (2014), who observed no symptoms in different eucalyptus clones after 28 days of exposure to auxinic herbicides, with no significant differences detected between the treatments.

TUFFI SANTOS et al. (2006) reported recovery of *Eucalyptus urophylla* plants at 15 and 30 DAA at the doses of 56.25% and 15.10%, respectively, of the herbicide triclopyr. According to EGAN et al. (2014), after being exposed to low doses of auxin-mimicking herbicides, certain plant species have the ability to recover from low to moderate injury symptoms.

Figure 6. Shoot emergence in *Eucalyptus urograndis* seedlings after exposure to 240 g ae ha^{-1} of the herbicide dicamba, at 28 DAA.

As can be seen in Figures 7A and 7B, the height and diameter variables of the eucalyptus stem were affected by the dicamba doses at 14 and 28 DAA. Plant height decreased linearly with the increasing doses, and this effect was most pronounced in the evaluation performed at 28 DAA, when plant height decreased by 0.15 cm per g ae ha^{-1} of dicamba applied (Figure 7A). Stem diameter also decreased linearly, by 0.0126 and 0.0195 mm, with each 1 g ae ha^{-1} of dicamba applied at 14 and 28 DAA, respectively (Figure 7B).

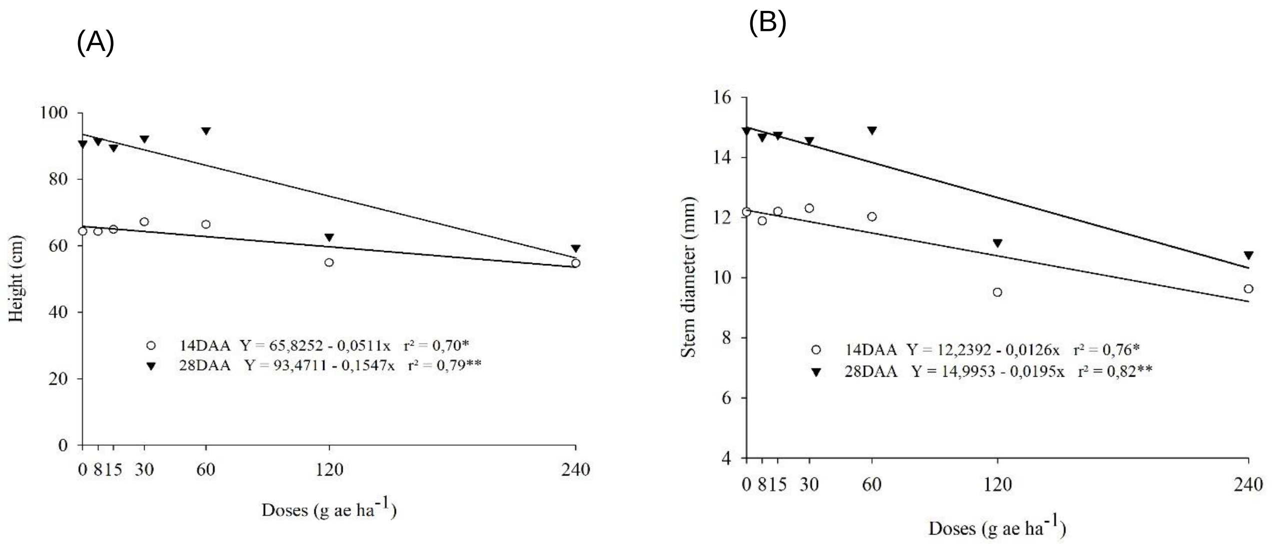

Figure 7. Plant height (A) and stem diameter (B) of *Eucalyptus urograndis* plants before application of the herbicide dicamba and at 14 and 28 days after application (DAA) of dicamba doses.

The number of branches and the dry mass of branches and leaf decreased linearly, by 0.03 branches and 0.36, 0.45 and 0.49 g per plant, respectively, with each 1 g ae ha^{-1} increase in the dicamba dose applied (Figures 8A, 8B, and 8C).

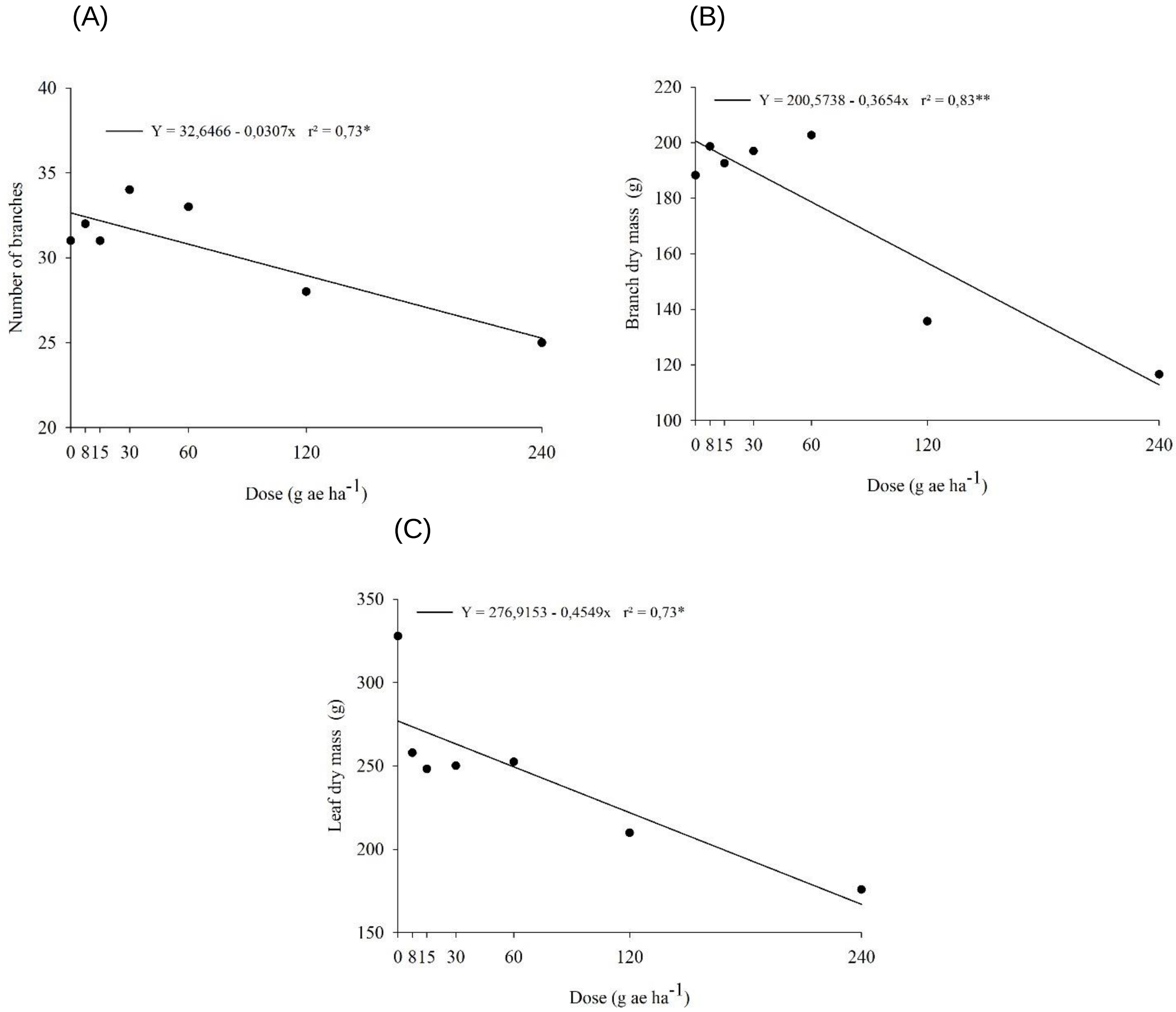

Figure 8. Number of branches (A) and dry mass of branches (B), leaves (C) and of *Eucalyptus urograndis* plants at 90 days after application of doses of the dicamba herbicide.

Negative effects with herbicide subdoses were also described by TUFFI SANTOS et al. (2006), who reported a reduction in the height and diameter of *Eucalyptus urophylla* plants after the application of glyphosate, triclopyr, carfentrazone and their mixtures. In their study, the authors observed a 22.5% reduction in dry mass accumulation after exposing eucalyptus plants to the auxinic herbicide triclopyr.

In their study, CARVALHO et al. (2014) showed that the subdoses of triclopyr and fluroxypyr + triclopyr negatively impacted the height, diameter and dry matter of different eucalyptus clones at 28 DAA. Additionally, the dry mass of the *Eucalyptus urograndis* hybrid decreased by 8.82%, on average, when compared with the control treatment. These results show that subdoses of auxinic herbicides were detrimental to the growth of the hybrid under study.

According to ROBINSON et al. (2013), reductions in biometric variables of different plant species after exposure to auxin-mimicking herbicides are due to the increase in abscisic acid (ABA) restricts plant growth for some time until the plant recovers. As stated by KELLEY et al. (2005), auxinic herbicides activate auxin-response genes, favoring excessive ethylene production and, later, of ABA, causing disorganized growth in the plant. D'ANTONINO et al. (2012) asserted that exposing sensitive plants to auxinic herbicides causes a reduction in growth rate, besides favoring disturbance of the photosynthetic apparatus and the metabolism of nucleic acids, resulting in the twisting of leaves and branches and death of the apical meristem, in addition to cell-wall loosening.

Inadequate growth and development of plants can lead, for instance, to a decrease in the number of branches and plant height, diameter, and leaf area, compromising photoassimilate production (SOLOMON &

BRADLEY 2014) and reducing the yield and quality of wood, in the case of eucalyptus. In addition, non-recovery or late recovery of these variables can compromise wood production throughout the crop cycle.

CONCLUSION

Dicamba doses greater than 120 g ae ha^{-1} cause injuries to young eucalyptus plants. The main symptoms are leaf epinasty, twisting of branches, shoot emergence and necrosis of meristematic tissues. Phytotoxicity by the herbicide at the doses of 120 and 240 g ae ha^{-1} affects the number of branches, plant height, stem diameter and the dry masses of branches and leaves. The results obtained in the present study are of practical importance for the chemical control of weeds.

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